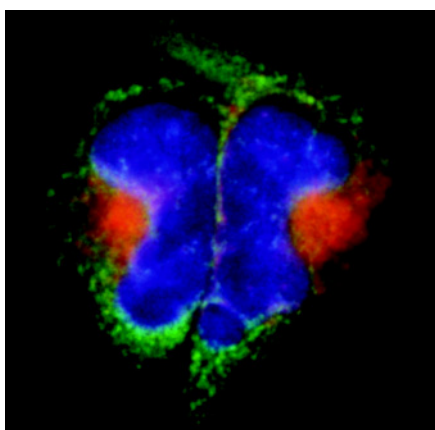


**Product List — 12.3 Probes for Lysosomes, Yeast Vacuoles and Other Acidic Organelles**

Cat #	Product Name	Unit Size
A-1301	acridine orange	1 g
A-3568	acridine orange *10 mg/mL solution in water*	10 mL
A-6458	anti-alkaline phosphatase (yeast vacuolar), mouse IgG <sub>1</sub> , monoclonal 1D3 *in conditioned culture medium*	2.5 mL
A-6428	anti-carboxypeptidase Y (yeast vacuolar), mouse IgG <sub>1</sub> , monoclonal 10A5 (anti-CPY)	250 µg
A-6422	anti-H <sup>+</sup> -ATPase 69 kDa subunit (yeast vacuolar), mouse IgG <sub>2a</sub> , monoclonal 8B1	250 µg
A-6426	anti-H <sup>+</sup> -ATPase 100 kDa subunit (yeast vacuolar), mouse IgG <sub>2a</sub> , monoclonal 10D7	250 µg
A-6427	anti-H <sup>+</sup> -ATPase 60 kDa subunit (yeast vacuolar), mouse IgG <sub>1</sub> , monoclonal 13D11	250 µg
A-21273	anti-Pep12p (yeast), mouse IgG <sub>1</sub> , monoclonal 2C3 *0.5 mg/mL*	100 µg
A-6457	anti-3-phosphoglycerate kinase (yeast), mouse IgG <sub>1</sub> , monoclonal 22C5 (anti-PGK)	250 µg
B-22461	BODIPY <sup>®</sup> FL histamine	1 mg
D-10460	Dapoxyl <sup>®</sup> (2-aminoethyl)sulfonamide	10 mg
D-113	5-dimethylaminonaphthalene-1-( <i>N</i> -(5-aminopentyl))sulfonamide (dansyl cadaverine)	100 mg
D-1552	<i>N</i> -(3-((2,4-dinitrophenyl)amino)propyl)- <i>N</i> -(3-aminopropyl)methylamine, dihydrochloride (DAMP)	100 mg
F-7030	FUN <sup>®</sup> 1 cell stain *10 mM solution in DMSO*	100 µL
F-13150	FUN <sup>®</sup> 2 cell stain *10 mM solution in DMSO*	100 µL
L-7533	LysoSensor <sup>™</sup> Blue DND-167 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-7534	LysoSensor <sup>™</sup> Green DND-153 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-7535	LysoSensor <sup>™</sup> Green DND-189 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-22460	LysoSensor <sup>™</sup> Yellow/Blue dextran, 10,000 MW, anionic, fixable	5 mg
L-7545	LysoSensor <sup>™</sup> Yellow/Blue DND-160 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-7525	LysoTracker <sup>®</sup> Blue DND-22 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-12490	LysoTracker <sup>®</sup> Blue-White DPX *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-7526	LysoTracker <sup>®</sup> Green DND-26 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-7528	LysoTracker <sup>®</sup> Red DND-99 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
L-12491	LysoTracker <sup>®</sup> Yellow HCK-123 *1 mM solution in DMSO* *special packaging*	20 x 50 µL
N-3246	neutral red *high purity*	25 mg
T-3166	<i>N</i> -(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM <sup>®</sup> 4-64)	1 mg
T-13320	<i>N</i> -(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM <sup>®</sup> 4-64) *special packaging*	10 x 100 µg
T-23360	<i>N</i> -(3-trimethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM <sup>®</sup> 5-95)	1 mg
Y-7531	Yeast Vacuole Marker Sampler Kit	1 kit
Y-7536	yeast vacuole membrane marker MDY-64	1 mg



**Figure 12.45** Fixed and permeabilized osteosarcoma cells simultaneously stained with the fluorescent lectins, Alexa Fluor 488 concanavalin A (Con A) (C-11252) and Alexa Fluor 594 wheat germ agglutinin (WGA) (W-11262). Con A selectively binds  $\alpha$ -glucopyranosyl residues, whereas WGA selectively binds sialic acid and *N*-acetylglucosaminyl residues. The nuclei were counterstained with blue-fluorescent Hoechst 33342 nucleic acid stain (H-1399, H-3570, H-21492). The image was acquired using bandpass filter sets appropriate for the Texas Red dye, fluorescein and AMCA.

## 12.4 Probes for the Endoplasmic Reticulum and Golgi Apparatus

The endoplasmic reticulum (ER) and Golgi apparatus (Figure 12.1) are primarily responsible for the proper sorting of lipids and proteins in cells.<sup>1</sup> Consequently, most of the cell-permeant probes for these organelles are either lipids or chemicals that affect protein movement. Several of the most effective probes for the Golgi apparatus are ceramides and sphingolipids, which are also discussed in Section 13.3. Aspects of lipid trafficking through these organelles that relate to signal transduction are described in Section 18.4. An excellent compendium of human diseases that affect intracellular transport processes through lysosomes, Golgi and endoplasmic reticulum (ER) has been published.<sup>2</sup> Enzymes in the ER are also involved in synthesis of cholesterol and membranes and in the detoxification of hydrophobic drugs through the cytochrome P-450 system (Section 10.6). Because several enzymes in the Golgi glycosylate lipids and proteins, some fluorescent lectins are useful markers for this organelle (Figure 12.45).

In both live and fixed cells, the flattened membranous sacs of the ER and the Golgi apparatus can be stained with a variety of lipophilic probes and then distinguished on the basis of their morphology. Alternatively, the Golgi apparatus can be selectively stained with one of the fluorescent ceramide analogs, which tend to associate preferentially with the trans-Golgi. The lipophilicity of these probes enables their facile loading into live cells, usually from a dilute solution in dimethylsulfoxide. Nissl bodies principally comprise ordered structures of alternate lamellae of rough endoplasmic reticulum and arrays of polyribosomes (Figure 8.37, Figure 14.1, Figure 14.40). Our NeuroTrace fluorescent Nissl stains are described in Section 14.3.

## ER-Tracker Blue-White DPX

ER-Tracker Blue-White DPX (E-12353, Figure 12.46) is a highly selective and photostable stain for the ER in live cells<sup>3–5</sup> (Figure 12.47). Unlike the conventional ER stain DiOC<sub>6</sub>(3), ER-Tracker Blue-White DPX does not stain mitochondria, and staining at low concentrations does not appear to be toxic to cells. Moreover, our experiments indicate that the staining patterns are retained after fixation with aldehydes, although at reduced intensity.

ER-Tracker Blue-White DPX is a member of our Dapoxyl dye family<sup>6</sup> and thus exhibits an unusually large Stokes shift and long-wavelength emission with a high extinction coefficient and high quantum yield when in a hydrophobic environment (Figure 13.48). Its fluorescence is highly environment sensitive — with increasing solvent polarity, the fluorescence maximum shifts to longer wavelengths and the quantum yield decreases — and peak fluorescence emission ranges from 430 nm to 640 nm. The ER-Tracker Blue-White DPX dye is readily visualized by two-photon microscopy.<sup>7</sup> We recommend visualizing its ER staining with a standard DAPI or UV longpass optical filter set (Table 24.8).

## Carbocyanine Dyes

### Short-Chain Carbocyanine Dyes

Terasaki and co-workers used the short-chain carbocyanine DiOC<sub>6</sub>(3) (D-273, Section 12.2) to visualize the ER in both live and aldehyde-fixed cells.<sup>8–10</sup> This dye and the similar DiOC<sub>5</sub>(3) (D-272) have since been used extensively to study structural interactions and dynamics of the ER in neurons,<sup>11,12</sup> yeast,<sup>13</sup> onion epidermis,<sup>14</sup> and to examine the morphological relationships between the ER, mitochondria, intermediate filaments and microtubules in various cell types.<sup>15–17</sup> DiOC<sub>6</sub>(3) and DiOC<sub>5</sub>(3) pass through the plasma membrane and stain intracellular membranes with a fluorescein-like fluorescence; ER membranes can easily be distinguished by their characteristic morphology.<sup>18</sup> Caution must be exercised, however, in using the carbocyanines as probes for the ER. It has been reported that ER staining with DiOC<sub>6</sub>(3) does not occur until the mitochondria round up and lose the fluorochrome.<sup>19</sup> Rhodamine 6G and the hexyl ester of rhodamine B (R-634, R-648; Section 12.2) appear to stain like DiOC<sub>6</sub>(3), except they are apparently less toxic and they fluoresce orange, providing possibilities for multicolor labeling.<sup>18,20</sup> When used at very low concentrations, these slightly lipophilic rhodamine dyes tend to stain only mitochondria of live cells.<sup>21</sup>

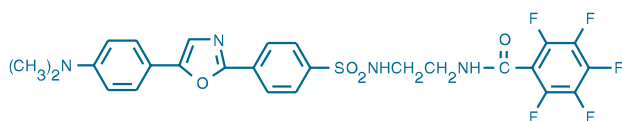


Figure 12.46 E-12353 ER-Tracker Blue-White DPX.

### Long-Chain Carbocyanine Dyes

Terasaki and Jaffe have used the long-chain carbocyanines DiIC<sub>16</sub>(3) and DiIC<sub>18</sub>(3) (D-384, D-282) to label ER membranes. They achieved selective labeling of the ER by microinjecting a saturated solution of DiI in oil into sea urchin eggs.<sup>22,23</sup> As noted in Section 13.4 on dialkylcarbocyanine and dialkylaminostyryl probes, DiI diffuses only in continuous membranes. This method has been successful in several other egg types but was not effective in molluscan or arthropod axons.

## Brefeldin A and Its Fluorescent Analogs

The fungal metabolite brefeldin A (BFA) has proven valuable for dissecting the cellular processes, including vesicle formation<sup>24</sup> and kinesin distribution,<sup>25</sup> involved in exporting newly synthesized proteins.<sup>26</sup> In addition to the natural product isolated from *Penicillium brefeldianum* (B-7450), we offer green- and orange-fluorescent BODIPY derivatives of brefeldin A<sup>3,5,27,28</sup> (B-7447, B-7449).

### Brefeldin A

BFA (B-7450) has multiple targets in cells.<sup>29</sup> Exposing cells to BFA causes a distortion in intracellular protein traffic from the ER to the Golgi apparatus and the eventual loss of Golgi apparatus morphology; removal of BFA completely reverses these effects.<sup>26,30–34</sup> BFA also alters the morphology of endosomes and lysosomes.<sup>35</sup> BFA has been used to prevent retinoic acid potentiation of immunotoxins,<sup>36</sup> to study translocation of proteins in polarized epithelial cells<sup>37</sup> and to investigate the regulation of ADP-ribosylation factor binding to the Golgi apparatus.<sup>38</sup> BFA action can be monitored using fluorescent endosomal markers such as lucifer yellow CH<sup>35</sup> (L-453, L-682, L-1177, L-12926; Section 14.3) and tetramethylrhodamine-labeled transferrin<sup>39</sup> (T-2872, Section 16.1). Researchers have also used BFA to detect the intracellular expression of cytokines.<sup>40,41</sup> BFA disrupts Golgi-mediated intracellular transport and allows cytokines to accumulate, producing an enhanced cytokine signal that can be detected by flow cytometry.

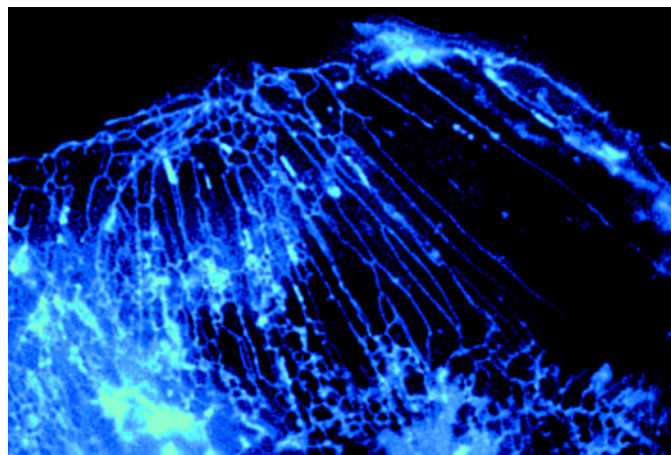


Figure 12.47 Live bovine pulmonary artery endothelial cells stained with ER-Tracker Blue-White DPX (E-12353), a Dapoxyl derivative. This image was acquired using a DAPI bandpass optical filter.

### Fluorescent Brefeldin A

The green-fluorescent BODIPY FL and red-orange-fluorescent BODIPY 558/568 BFA derivatives (B-7447, B-7449; Figure 12.48) are selectively localized in the ER and Golgi apparatus in four different cell lines.<sup>28</sup> BODIPY 558/568 BFA may be used in conjunction with NBD C<sub>6</sub>-ceramide (N-1154) to investigate the ER and Golgi apparatus simultaneously. BODIPY 558/568 has also been used in combination with ER Tracker Blue-White DPX to label the endoplasmic reticulum of hyphae.<sup>5</sup> However, the biological activity of the fluorescent BFA derivatives may be limited and may be dependent on cleavage of the BODIPY fluorophore from BFA by intracellular esterases.<sup>28</sup> Two isomeric esters are isolated in the synthesis of the fluorescent brefeldins; we are selling only “isomer 1” of each product.

### Fluorescent Ceramide Analogs

NBD C<sub>6</sub>-ceramide (N-1154) and BODIPY FL C<sub>5</sub>-ceramide (D-3521), both of which can be used with fluorescein optical filter sets (Table 24.8), are selective stains for the Golgi apparatus.<sup>42–44</sup> With spectral properties similar to those of Texas Red dye, BODIPY TR ceramide<sup>45,46</sup> (D-7540) is especially useful for double-labeling experiments, including with chimeras of green-fluorescent proteins,<sup>47,48</sup> as well as for staining cells and tissues that have substantial amounts of green autofluorescence. In addition, the BODIPY TR fluorophore is ideal for imaging microscopy with CCD cameras or other red-sensitive detectors.

#### NBD C<sub>6</sub>-Ceramide and NBD C<sub>6</sub>-Sphingomyelin

NBD C<sub>6</sub>-ceramide (N-1154) has been used extensively as a selective stain of the trans-Golgi in both live and fixed cells.<sup>49–56</sup> Complexing fluorescent ceramides with serum albumin (BSA) facilitates cell labeling without requiring the use of organic solvents to dissolve the probe.<sup>42</sup> For this application, we provide NBD C<sub>6</sub>-ceramide complexed with defatted BSA (N-22651). Researchers have employed NBD C<sub>6</sub>-ceramide to investigate:

- Defective trans-Golgi acidification in cystic fibrosis<sup>57</sup>
- Effects of BFA (B-7450) on the transport of proteins from the Golgi apparatus to the ER<sup>30,32,58</sup>

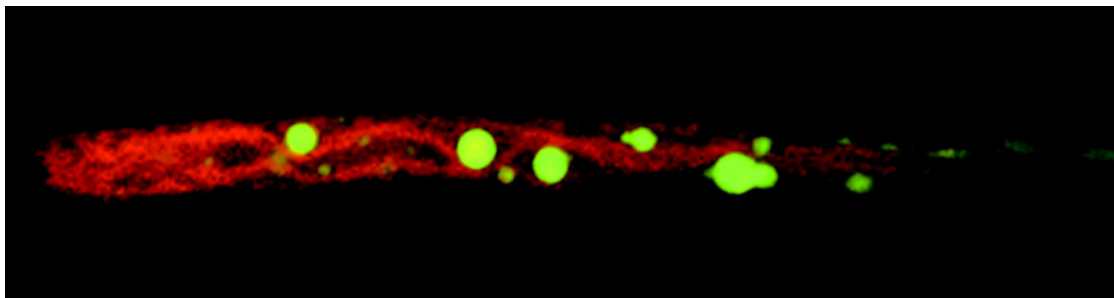
- Farber’s disease, a genetically inherited disease of lipid metabolism<sup>59</sup>
- Inhibition of glycoprotein traffic through secretory pathways<sup>60</sup>
- Intracellular trafficking and targeting of thrombin receptors<sup>61</sup>
- Secretory activity during the isolation of secretion mutants by fluorescence-activated cell sorting<sup>62</sup>
- Subcellular distribution of the verotoxin B subunit in Vero cells<sup>63</sup>

Furthermore, the fluorescence of NBD C<sub>6</sub>-ceramide is apparently sensitive to the cholesterol content of the Golgi apparatus, a phenomenon that is not observed with BODIPY FL C<sub>5</sub>-ceramide.<sup>64</sup> If NBD C<sub>6</sub>-ceramide-containing cells are starved for cholesterol, the NBD C<sub>6</sub>-ceramide that accumulates within the Golgi apparatus appears to be severely photolabile. However, this NBD photobleaching can be reduced by stimulation of cholesterol synthesis. Thus, NBD C<sub>6</sub>-ceramide may be useful in monitoring the cholesterol content of the Golgi apparatus in live cells.<sup>64</sup>

NBD C<sub>6</sub>-ceramide’s conversion to the NBD C<sub>6</sub>-glycosyl ceramide and NBD C<sub>6</sub>-sphingomyelin (N-3524) has been observed *in vivo*.<sup>65–67</sup> Metabolism of the probe in live Chinese hamster ovary (CHO) fibroblasts has been used to define lipid-transport pathways.<sup>65,68</sup> NBD C<sub>6</sub>-ceramide is reported to be metabolized to NBD C<sub>6</sub>-sphingomyelin in *Plasmodium falciparum*-infected erythrocytes, but not in normal erythrocytes.<sup>69,70</sup> Like NBD C<sub>6</sub>-ceramide, NBD C<sub>6</sub>-sphingomyelin has been used for the study of lipid trafficking between organelles.<sup>68,71,72</sup> Normal fibroblasts hydrolyze NBD C<sub>6</sub>-sphingomyelin and process it through the Golgi apparatus.<sup>73</sup> However, in human skin fibroblasts from patients with Niemann–Pick disease, which is characterized by a lack of lysosomal sphingomyelinase activity, NBD C<sub>6</sub>-sphingomyelin accumulates in the lysosomes.

#### BODIPY Ceramides and BODIPY Sphingomyelin

The green-fluorescent BODIPY FL C<sub>5</sub>-ceramide (D-3521) is more fade-resistant and brighter than the NBD derivative and can likely be substituted for the NBD C<sub>6</sub>-ceramide in many of its applications. As with NBD C<sub>6</sub>-ceramide, we also offer BODIPY FL C<sub>5</sub>-ceramide complexed with defatted BSA (B-22650) to facilitate cell labeling without the use of organic solvents to



**Figure 12.48** Endoplasmic reticulum (ER) of the hyphal tip cells of *Pisolithus tinctorius* were labeled with the red-orange-fluorescent BODIPY 558/568 conjugate of brefeldin A (B-7449), a reversible inhibitor of protein transport from the ER to the Golgi apparatus in many cell types and spe-

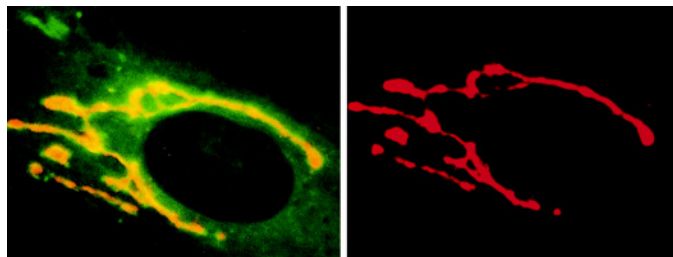
cies. The motile tubular vacuole system was stained with green-fluorescent carboxy-DFFDA (Oregon Green 488 carboxylic acid diacetate, O-6151). Image contributed by Danielle Davies, Department of Biological Sciences, University of New South Wales, Sydney, Australia.

dissolve the probe.<sup>42</sup> The red-fluorescent BODIPY TR ceramide (D-7540) has proven useful for two-color immunofluorescence using a fluorescein-labeled antibody.<sup>74</sup>

During normal resting intracellular transport, the kinetics of dye loading and transport may differ somewhat between the BODIPY and NBD analogs.<sup>75</sup> BODIPY FL C<sub>5</sub>-ceramide has proven to be an excellent structural marker for the Golgi apparatus, visualized either by fluorescence microscopy<sup>76,77</sup> or, following diaminobenzidine (DAB) conversion, electron microscopy.<sup>78–80</sup> BODIPY FL C<sub>5</sub>-ceramide has also been used to:

- Delineate the Golgi apparatus in the cytoarchitecture of size-excluding compartments in live cells<sup>81</sup>
- Investigate both the inhibition of glycoprotein transport by ceramides<sup>60</sup> and the possible link between protein secretory pathways and sphingolipid biosynthesis<sup>82</sup>
- Isolate mammalian secretion mutants<sup>62</sup>
- Study sphingolipid distribution during human keratinocyte differentiation<sup>83</sup>
- Visualize tubovesicular membranes induced by *Plasmodium falciparum*<sup>69,84</sup>

BODIPY FL C<sub>5</sub>-ceramide exhibits concentration-dependent fluorescence properties that provide additional benefits for imaging the Golgi apparatus. At high concentrations, the nonpolar BODIPY FL fluorophore forms excimers, resulting in a shift of the fluorophore's emission maximum from 515 nm (green) to ~620 nm (red). BODIPY FL C<sub>5</sub>-ceramide accumulation is sufficient for excimer formation in the trans-Golgi but not in the surrounding cytoplasm. Longpass optical filters that isolate the red emission can thus be used to selectively visualize the Golgi apparatus (Figure 12.49, Figure 12.50). Moreover, this two-color property can be used to quantitate BODIPY FL C<sub>5</sub>-ceramide accumulation by ratio imaging.<sup>43,82,85</sup> Like BODIPY FL C<sub>5</sub>-ceramide, the red-fluorescent BODIPY TR ceramide appears to form long-wavelength excimers when concentrated in the Golgi apparatus; in this case, however, the excimers exhibit infrared fluorescence. In an unexpected application, it has been shown that cells infected with some intracellular bacteria, including *Chlamydia psittaci*, accumulate BODIPY FL C<sub>5</sub>-ceramide (D-3521, Section 12.4) in their inclusion membranes rather than in the Golgi of the host cells.<sup>86,87</sup> Certain CellTracker reagents (Section 14.2) that were used in combination with BODIPY FL C<sub>5</sub>-ceramide were also found to selectively label intracellular bacteria and parasites.<sup>86</sup>

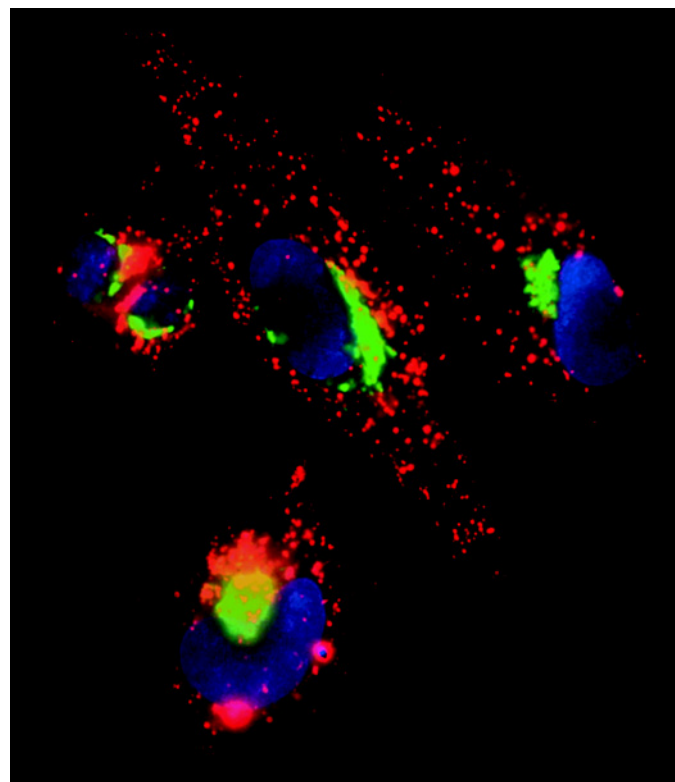


**Figure 12.49** Selective staining of the Golgi apparatus using the green-fluorescent BODIPY FL C<sub>5</sub>-ceramide (D-3521) (left panel). At high concentrations, the BODIPY FL fluorophore forms excimers that can be visualized using a red long-pass optical filter. The BODIPY FL C<sub>5</sub>-ceramide accumulation in the trans-Golgi is sufficient for excimer formation (right panel) (J Cell Biol 113, 1267 (1991)). Images contributed by Richard Pagano, Mayo Foundation.

We also offer BODIPY FL C<sub>5</sub>-sphingomyelin (D-3522) — the likely metabolic product of BODIPY FL C<sub>5</sub>-ceramide<sup>43</sup> — as well as BODIPY FL C<sub>12</sub>-sphingomyelin<sup>88</sup> (D-7711) and BODIPY FL C<sub>5</sub>- and C<sub>12</sub>-glucocerebrosides (D-7548, D-7547; Section 13.3). The concentration-dependent fluorescence shift of BODIPY FL C<sub>5</sub>-sphingomyelin from green to red has been used to follow the initial steps of lipid uptake and transport by early endosomes through the cytoplasm.<sup>89</sup> BODIPY FL C<sub>5</sub>-glucocerebroside is reportedly internalized by endocytic and nonendocytic pathways that are quite different from those governing the internalization of BODIPY FL C<sub>5</sub>-sphingomyelin<sup>90</sup> (D-3522). Pagano and collaborators have published reviews of the use of BODIPY ceramides and BODIPY sphingolipids to study the endocytic pathway in mammalian cells.<sup>42,91</sup>

### Monoclonal Antibody Specific for the Yeast Endoplasmic Reticulum

For detection of yeast endoplasmic reticulum membranes, we offer the anti-dolichol phosphate mannose synthase (Dol-P-Man synthase, Dpm1p) monoclonal antibody<sup>92</sup> (A-6429). The yeast Dol-P-Man synthase is an ~30,000-dalton integral membrane protein that resides in the endoplasmic reticulum;<sup>93</sup> the monoclonal antibody was prepared against the cytosolic domain of the protein.

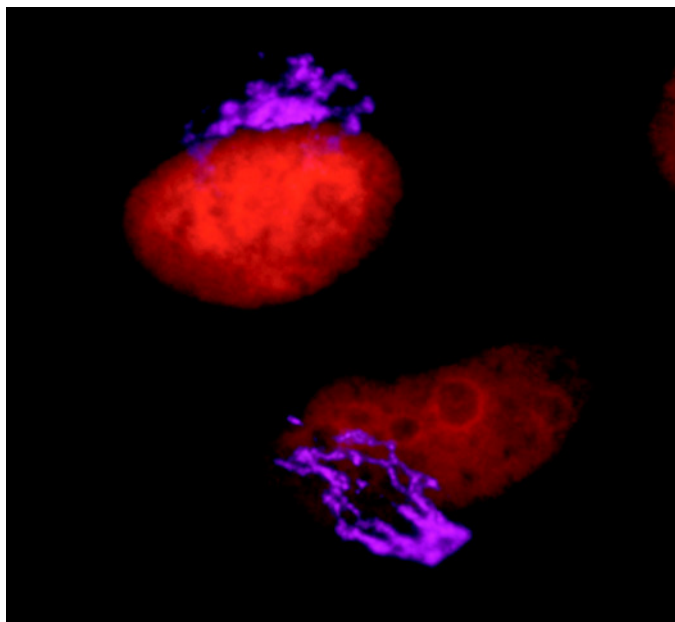


**Figure 12.50** Viable Madin-Darby canine kidney (MDCK) cells sequentially stained with BODIPY FL C<sub>5</sub>-ceramide (D-3521, B-22650), LysoTracker Red DND-99 (L-7528) and Hoechst 33258 (H-1398, H-3569, H-21491). Green-fluorescent BODIPY FL C<sub>5</sub>-ceramide localized to the Golgi apparatus, red-fluorescent LysoTracker Red stain accumulated in the lysosomes, and the blue-fluorescent Hoechst 33258 dye stained the nuclei. The multiple-exposure image was acquired with bandpass filters appropriate for fluorescein, Texas Red dye and DAPI.

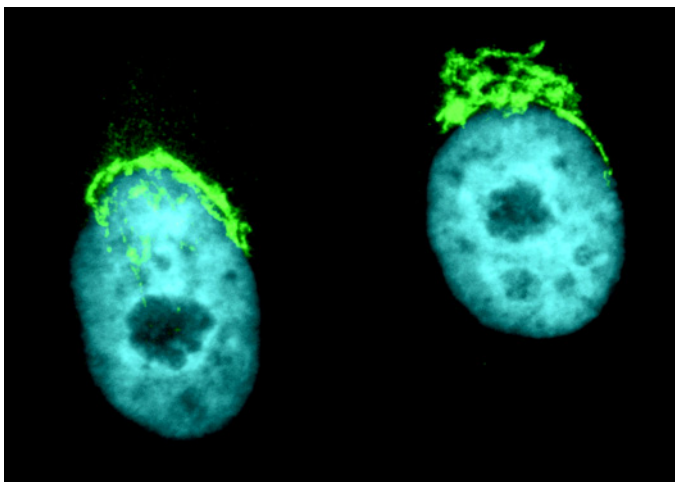
## Antibodies to Mammalian and Yeast Golgi Proteins

### Anti-Human Golgin-97

Originally isolated from the serum of a patient with the autoimmune disease known as Sjögren's syndrome, anti-human golgin-97 antibodies recognize a 97,000-dalton protein called golgin-97, a member of the granin family of proteins and a peripheral membrane protein localized on the cytoplasmic face of the Golgi apparatus.<sup>94</sup> Because the antibody recognizes a protein unique to the Golgi apparatus of most vertebrate species, it is a



**Figure 12.51** Fixed, permeabilized HeLa cells were labeled with anti-golgin-97 antibody (A-21270) and detected using Alexa Fluor 647 goat anti-mouse IgG antibody (A-21235). The cells were counterstained with SYTOX Orange nucleic acid stain (S-11368).



**Figure 12.52** Fixed, permeabilized HeLa cells were labeled with anti-golgin-97 antibody (A-21270) and detected using Alexa Fluor 488 goat anti-mouse IgG antibody (A-11001). The cells were counterstained with DAPI (D-1306, D-3571, D-21490). The image was deconvolved using Huygens software (Scientific Volume Imaging, www.svi.nl). 3-D reconstruction was performed using Imaris software (Bitplane AG).

useful for immunodetection and identification of the Golgi apparatus in cells, Western blotting and immunoprecipitation; however, a customer reports that it may not work for rat cells (Figure 12.5, Figure 12.51, Figure 12.52). Molecular Probes offers anti-human golgin-97, mouse IgG<sub>1</sub> isotype monoclonal antibody CDF4 (A-21270).

### Monoclonal Antibody Specific for the Yeast Late-Golgi Compartment

For detection of the late-Golgi compartment of yeast, we offer a mouse monoclonal antibody to Vps10p (A-21274). Yeast Vps10p is an ~180,000-dalton membrane protein that resides in the late-Golgi compartment. This monoclonal antibody is a valuable tool for detecting the late-Golgi compartment and late-Golgi membranes in yeast subcellular fractions by Western blot. However, due to the low abundance of the Vps10p antigen, this monoclonal antibody cannot be used to visualize Golgi by immunocytochemistry unless the signal is amplified, such as with our tyramide signal-amplification (TSA) technology (Section 6.2).

## Lectins for Staining the Golgi Apparatus

### Wheat Germ Agglutinin and Concanavalin A

Various proteins and lipids found in the Golgi apparatus are glycosylated. Consequently, lectin conjugates (Section 7.7) have been found to be particularly useful for staining Golgi structures in fixed-cell preparations (Figure 7.96). Wheat germ agglutinin (WGA) staining of trans-Golgi has been considered a marker for this organelle.<sup>95–97</sup> Fluorescent conjugates of concanavalin A (Con A) also stain the Golgi but with reduced specificity.<sup>98</sup> Molecular Probes prepares WGA and Con A conjugates whose fluorescence spans the entire visible and near-IR spectrum (Table 7.18). Our Alexa Fluor conjugates of these important lectins are particularly recommended for their enhanced brightness and photostability. We also offer a Wheat Germ Agglutinin Stain Sampler Kit (W-7024), which contains 1 mg quantities each of WGA conjugates of the Alexa Fluor 350, Oregon Green 488, tetramethylrhodamine and Texas Red-X dyes.

### Griffonia simplicifolia Lectin GS-II

Lectin GS-II from *Griffonia simplicifolia* is the only known lectin that binds with high selectivity to terminal, nonreducing  $\alpha$ - and  $\beta$ -*N*-acetyl-D-glucosaminyl (GlcNAc) residues of glycoproteins. Because of the affinity of lectin GS-II for GlcNAc, conjugates of this lectin are useful for staining intermediate-to-trans Golgi — the site of *N*-acetylglucosaminyltransferase activity.<sup>99</sup> The Golgi apparatus of oligodendrocytes and ganglion neurons are readily stained by fluorescent GS-II conjugates. We have prepared the green-fluorescent Alexa Fluor 488 conjugate of lectin GS-II (L-21415) for use in Golgi staining.

### Helix pomatia (Edible Snail) Agglutinin

*Helix pomatia* agglutinin (HPA) selectively binds to terminal  $\alpha$ -*N*-acetylgalactosaminyl residues — an intermediate sugar added in *O*-linkage to serine and threonine residues in *cis*-Golgi cisternae and then substituted with galactose and sialic acid in the *trans*-Golgi.<sup>100</sup> HPA conjugates are principally used as markers for the Golgi. Our green-fluorescent Alexa Fluor 488 conjugate of HPA (L-11271) should be a particularly useful probe for Golgi staining.

## References

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*The full citations and, in most cases, links to PubMed for all references in this Handbook are available at our Web site ([www.probes.com/search](http://www.probes.com/search)).*

## Data Table — 12.4 Probes for the Endoplasmic Reticulum and Golgi Apparatus

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
B-7447	554.44	F,D,L	DMSO, EtOH	503	83,000	510	MeOH	
B-7449	608.51	F,D,L	DMSO, EtOH	559	80,000	568	MeOH	
B-7450	280.36	F,D	DMSO, EtOH	<300		none		
B-22650	see Notes	F,D,L	H <sub>2</sub> O	505	91,000	511	MeOH	1, 2
D-272	544.47	D,L	DMSO	484	155,000	500	MeOH	
D-282	933.88	L	DMSO, EtOH	549	148,000	565	MeOH	
D-384	877.77	L	DMSO, EtOH	549	148,000	565	MeOH	
D-3521	601.63	FF,D,L	CHCl <sub>3</sub> , DMSO	505	91,000	511	MeOH	1
D-3522	766.75	FF,D,L	CHCl <sub>3</sub> , DMSO	505	77,000	512	MeOH	1
D-7540	705.71	FF,D,L	CHCl <sub>3</sub> , DMSO	589	60,000	617	MeOH	
D-7711	864.94	FF,D,L	DMSO	505	75,000	513	MeOH	1, 3
E-12353	580.53	F,D,L	DMSO	374	25,000	575	MeOH	3, 4
N-1154	575.75	FF,D,L	CHCl <sub>3</sub> , DMSO	466	22,000	536	MeOH	5
N-3524	740.88	FF,D,L	CHCl <sub>3</sub> , DMSO	466	22,000	536	MeOH	5
N-22651	see Notes	F,D,L	H <sub>2</sub> O	466	22,000	536	MeOH	2, 5

For definitions of the contents of this data table, see "How to Use This Book" on page viii.

### Notes

- Em for BODIPY FL sphingolipids shifts to ~620 nm when high concentrations of the probe (>5 mol%) are incorporated in lipid mixtures (*J Cell Biol* 113, 1267 (1991)).
- This product is a lipid complexed with bovine serum albumin (BSA; MW ~66,000). Spectroscopic data are for the free lipid in MeOH.
- This product is supplied as a ready-made solution in the solvent indicated under **Soluble**.
- ER-Tracker Blue-White DPX Abs = 379 nm, Em = 520 nm bound to phospholipid bilayer membranes. Fluorescence in water is very weak.
- NBD derivatives are almost nonfluorescent in water. QY and  $\tau$  increase and Em decreases in aprotic solvents and other nonpolar environments relative to water (*Biochemistry* 16, 5150 (1977); *Photochem Photobiol* 54, 361 (1991)).

**Product List — 12.4 Probes for the Endoplasmic Reticulum and Golgi Apparatus**

Cat #	Product Name	Unit Size
A-6429	anti-dolichol phosphate mannose synthase (yeast), mouse IgG <sub>1</sub> , monoclonal 5C5	250 µg
A-21270	anti-golgin-97 (human), mouse IgG <sub>1</sub> , monoclonal CDF4 (anti-Golgi)	100 µg
A-21273	anti-Pep12p (yeast), mouse IgG <sub>1</sub> , monoclonal 2C3 *0.5 mg/mL*	100 µL
A-21274	anti-Vps10p (yeast), mouse IgG <sub>2a</sub> , monoclonal 18C8	500 µg
B-22650	BODIPY® FL C <sub>5</sub> -ceramide complexed to BSA	5 mg
B-7450	brefeldin A *from <i>Penicillium brefeldianum</i> *	5 mg
B-7449	brefeldin A, BODIPY® 558/568 conjugate *isomer 1*	25 µg
B-7447	brefeldin A, BODIPY® FL conjugate *isomer 1*	25 µg
D-7711	<i>N</i> -(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza- <i>s</i> -indacene-3-dodecanoyl)sphingosyl phosphocholine (BODIPY® FL C <sub>12</sub> -sphingomyelin) *1 mg/mL in DMSO*	250 µL
D-3521	<i>N</i> -(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza- <i>s</i> -indacene-3-pentanoyl)sphingosine (BODIPY® FL C <sub>5</sub> -ceramide)	250 µg
D-3522	<i>N</i> -(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza- <i>s</i> -indacene-3-pentanoyl)sphingosyl phosphocholine (BODIPY® FL C <sub>5</sub> -sphingomyelin)	250 µg
D-7540	<i>N</i> -((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza- <i>s</i> -indacene-3-yl)phenoxy)acetyl)sphingosine (BODIPY® TR ceramide)	250 µg
D-384	1,1'-dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC <sub>16</sub> (3))	100 mg
D-282	1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate ('DiI'; DiIC <sub>18</sub> (3))	100 mg
D-272	3,3'-dipentylloxycarbocyanine iodide (DiOC <sub>5</sub> (3))	100 mg
E-12353	ER-Tracker™ Blue-White DPX *1 mM solution in DMSO* *special packaging*	20 x 50 µL
I-21410	isolectin GS-IB <sub>4</sub> from <i>Griffonia simplicifolia</i>	1 mg
I-21411	isolectin GS-IB <sub>4</sub> from <i>Griffonia simplicifolia</i> , Alexa Fluor® 488 conjugate	500 µg
I-21412	isolectin GS-IB <sub>4</sub> from <i>Griffonia simplicifolia</i> , Alexa Fluor® 568 conjugate	500 µg
I-21413	isolectin GS-IB <sub>4</sub> from <i>Griffonia simplicifolia</i> , Alexa Fluor® 594 conjugate	500 µg
I-21414	isolectin GS-IB <sub>4</sub> from <i>Griffonia simplicifolia</i> , biotin-XX conjugate	500 µg
L-21415	lectin GS-II from <i>Griffonia simplicifolia</i> , Alexa Fluor® 488 conjugate	500 µg
L-21416	lectin GS-II from <i>Griffonia simplicifolia</i> , Alexa Fluor® 594 conjugate	500 µg
L-11271	lectin HPA from <i>Helix pomatia</i> (edible snail), Alexa Fluor® 488 conjugate	1 mg
N-22651	NBD C <sub>6</sub> -ceramide complexed to BSA	5 mg
N-1154	6-(( <i>N</i> -(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoyl)sphingosine (NBD C <sub>6</sub> -ceramide)	1 mg
N-3524	6-(( <i>N</i> -(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoyl)sphingosyl phosphocholine (NBD C <sub>6</sub> -sphingomyelin)	1 mg
W-11263	wheat germ agglutinin, Alexa Fluor® 350 conjugate	5 mg
W-11261	wheat germ agglutinin, Alexa Fluor® 488 conjugate	5 mg
W-11262	wheat germ agglutinin, Alexa Fluor® 594 conjugate	5 mg
W-21404	wheat germ agglutinin, Alexa Fluor® 633 conjugate	5 mg
W-21407	wheat germ agglutinin, Alexa Fluor® 660 conjugate	5 mg
W-834	wheat germ agglutinin, fluorescein conjugate	5 mg
W-11260	wheat germ agglutinin, Marina Blue® conjugate	5 mg
W-6748	wheat germ agglutinin, Oregon Green® 488 conjugate	5 mg
W-7024	Wheat Germ Agglutinin Sampler Kit *four fluorescent conjugates, 1 mg each*	1 kit
W-849	wheat germ agglutinin, tetramethylrhodamine conjugate	5 mg
W-21405	wheat germ agglutinin, Texas Red®-X conjugate	1 mg

### Antifade Kits

Photobleaching is an inherent problem in fluorescence microscopy that often restricts the time available for observation and recording of images, and diminishes their signal-to-noise characteristics. Except in experiments using living specimens, addition of protective antifade reagents is the most straightforward practical measure for counteracting photobleaching, as it does not require tradeoffs between imaging setup parameters. Our ProLong, *SlowFade* and *SlowFade Light* Antifade Kits (see Section 24.1) offer proven effectiveness and a choice of solid-setting or liquid media formulations. The ProLong reagent is a two-component solid-setting medium that provides the most consistent antifade protection with minimal quenching across the widest range of dyes and probes. The *SlowFade* and *SlowFade Light* reagents are ready-to-use liquid media provided in convenient dropper bottles. The *SlowFade* and *SlowFade Light* reagents are differentiated by the extent to which they quench fluorescence and the duration of their protective effects.

